

Appl. No. 10/276,452  
Amdt. dated February 3, 2005  
Reply to Office action of October 5, 2004

Docket No. 58009-010600

### AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

#### Listing of Claims:

Claim 1-12 (canceled)

**Claim 13 (currently amended)** ~~A method of forming tissue on a bio-membrane using~~Use of crystalline beta-form fibroin for the production of a substrate according to anyone of the preceding claims, said substrate being suitable to allow for the survival, the proliferation and the correct differentiation and functioning of specialisedspecialized tissue cells of the human body both inside and outside of it, comprising:

seeding a plurality of human tissue cells on a bio-membrane, the bio-membrane including crystalline beta-form fibroin;

permitting the plurality of human tissue cells to proliferate;

permitting the plurality of human cells to differentiate; and

forming tissue on the bio-membrane.

**Claim 14 (currently amended)** ~~Use of fibroin according to~~The method of claim 1213, wherein the plurality of human body tissue cells is a ~~include those of the~~plurality of skin cells, and of the a plurality of liver cells, a plurality of mesothelial cells, a plurality of astrocytes, a plurality of human skeleton osteoblasts, a plurality of tenocytes, ~~or~~a plurality of human tendon fibroblasts, a plurality of chondrocytes, ~~or~~ a plurality of cells isolated from cartilaginous tissues, a plurality of endothelial cells of blood vessels, a plurality of steroid-secreting cells of the adrenal cortex, a plurality of smooth muscle cells of the *tunica muscularis* of the intestine and blood vessels, a plurality of squamous epithelial cells of the oral cavity, a plurality of squamous epithelial cells of ~~or~~ the conjunctiva/cornea and ~~or~~ a plurality of human pre-adipocytes of the white adipose connective tissue.

✓ Appl. No. 10/276,452

Amdt. dated February 3, 2005

Docket No. 58009-010600

Reply to Office action of October 5, 2004

**Claim 15 (currently amended)** ~~Process for the~~ A method of production of a substrate according to anyone of the preceding claims, comprising:

dissolving wherein degummed silk is dissolved in a solution of lithium bromide in water at a temperature higher than room temperature and at a standard pressure so as to obtain a dissolved solution; ~~the solution so obtained being then filtered~~

filtering the dissolved solution through a porous ceramic filter; ~~diluted~~

diluting the dissolved solution with distilled water; ~~dialysed and left~~

dialyzing the diluted solution using a membrane with a 3500 molecular weight cut-off so as to obtain a dialyzed solution;

permitting the dialyzed solution to evaporate in polystyrene containers as to obtain a membrane; ~~wherein the membrane so obtained is immersed; and~~

immersing the membrane in a solution of methanol and water to make it crystalline and hence insoluble in water.

**Claim 16 (new)** The method of claim 13, wherein the plurality of human tissue cells originate from one human body.

**Claim 17 (new)** The method of claim 13, wherein the plurality of human tissue cells originate from a plurality of human bodies.

**Claim 18 (new)** The method of claim 13, further comprising:

providing glucose to the plurality of human tissue cells; and

permitting the plurality of human tissue cells to consume the glucose and secrete lactic acid.

**Claim 19 (new)** The method of claim 13, wherein the plurality of human tissue cells includes fibroblasts, and further comprising allowing the fibroblasts to secrete extracellular matrix components and precursors to collagen fibers.

**Claim 20 (new)** The method of claim 13, wherein the plurality of human tissue cells are keratinocytes, and further comprising permitting the keratinocytes to form epithelium.

Appl. No. 10/276,452

Amdt. dated February 3, 2005

Docket No. 58009-010600

Reply to Office action of October 5, 2004

**Claim 21 (new)** The method of claim 13, wherein the plurality of human tissue cells includes pre-irradiated fibroblasts and keratinocytes, and further comprising allowing the cells to proliferate and function with normal cytological features without an occurrence of diffpoptosis

**Claim 22 (new)** The method according to claim 13, wherein said fibroin is secreted by the *Bombyx mori* silkworm.

**Claim 23 (new)** The method according to claim 13, wherein the bio-membrane includes comprises a polymer of natural origin.

**Claim 24 (new)** The method according to claim 13, wherein the bio-membrane includes a polymer of synthetic origin.

**Claim 25 (new)** The method according to claim 13, wherein the fibroin in the bio-membrane is present in a quantity varying from 20% to 80% in weight.

**Claim 26 (new)** A method of production of a substrate, comprising:

dissolving crystalline beta form fibroin in a solution of lithium bromide in water at a temperature higher than room temperature and at a standard pressure so as to obtain a dissolved solution;

filtering the dissolved solution through a porous ceramic filter;

diluting the dissolved solution with distilled water;

dialyzing the diluted solution using a membrane with a 3500 molecular weight cut-off so as to obtain a dialyzed solution;

permitting the dialyzed solution to evaporate in polystyrene containers as to obtain a membrane; and

immersing the membrane in a solution of methanol and water to make it crystalline and insoluble in water.